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Hereditary Nonpolyposis Colorectal Cancer: Review of Clinical, Molecular Genetics, and Counseling Aspects

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Lynch syndrome, or hereditary nonpolyposis colon cancer (HNPCC), is an autosomal-dominant disease accounting for approximately 1–5% of all colorectal cancer cases. Due to the lack of pathognomonic morphological or biomolecular markers, HNPCC has traditionally posed unique problems to clinicians and geneticists alike, both in terms of diagnosis and clinical management. Recently, novel insight into the pathogenesis of this syndrome has been provided by the identification of its molecular basis. In HNPCC families, germline mutations in any of four genes encoding proteins of a specialized DNA repair system, the mismatch repair, predispose to cancer development. Mutations in mismatch repair genes lead to an overall increase of the mutation rate and are associated with a phenotype of length instability of microsatellite loci. The present report summarizes the clinicopathological aspects of HNPCC and reviews the most recent molecular and biochemical findings.

KEY WORDS: colorectal cancer, mismatch repair, hereditary tumors

INTRODUCTION

In spite of the most recent advances in basic sciences [110], techniques of early diagnosis [46], and surgical approach [22], colorectal cancer (CRC) continues to be one of the major determinants of neoplastic morbidity and mortality in Western and industrialized countries [3].

The pathogenesis of CRC is of particular interest for the complex and only partially understood interaction between environmental factors (mostly related to diet and lifestyle) and genetic background. There are at least four aspects which make these neoplasms rather peculiar when compared to other cancer types. A first major point is the importance of exogenous factors in CRC development, as documented by numerous and consistent migration studies [24,64]. A second point is the existence of well-known lesions, the adenomatous polyps, which may appear several years before cancer and that can be easily removed during a routine endoscopy [76,86]. Third, a definite fraction of colorectal tumors is inherited through a Mendelian pattern of genetic transmission; this includes not only familial adenomatous polyposis (FAP) (or adenomatosis coli), in which the large bowel is carpeted by hundreds or thousands of polyps, but also the much more frequent hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome, in which tumors usually develop before age 45–50 years and are more often located in the proximal colon, without diffuse polyposis [57]. A fourth point of great importance concerns the molecular aspects of colorectal tumorigenesis. Several investigations have shown that the various steps of tumor development are closely associated with the accumulation of genetic errors, which involve both activation of dominant oncogenes and loss of function of tumor suppressor genes [17,99,109]. The gene responsible for FAP was recently mapped and cloned from chromosome 5q [33,37,44], and even more recently at least four genes have been identified (designated as *hMSH2, hMLH1, hPMS1,* and *hPMS2*) which are involved in DNA mismatch repair and which were found to be mutated in HNPCC [8,18,43,48,77,80,82].

The main purpose of the present review is to summarize the most relevant epidemiological, clinical, and biomolecular aspects of HNPCC, with particular emphasis on the existing difficulties and controversies regarding clinical recognition of the syndrome, and on the most recent genetic findings.

DEFINITION OF HNPCC

HNPCC is an autosomal-dominant disorder characterized by early appearance of tumors, predominantly localized in the proximal colon and occasionally multiple, and frequently associated with tumors of other or-

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gans. The disease was described for the first time in 1913 by A.S. Warthin, who reported on some families with multiple occurrence of gastric and colorectal carcinomas spanning three generations [111]. Some of these families were "revisited" in more recent years by Lynch et al., who described in more detail the main characteristics of the disease [54,58].

HNPCC is characterized by the appearance of cancer usually before the age of 45–50 years. Tumors of the large bowel are by far the most common malignancies, and in about 70% of patients they are located in the proximal colon and tend to be multifocal. Colorectal polyps are commonly observed in HNPCC, although it is still uncertain whether these lesions are more frequent than in the general population or are histologically more malignant [31,41,70]. Tumors of other organs are also frequently observed.

HNPCC is usually divided into two clinical forms. In Lynch syndrome type I, CRC is the only cancer observed, and in Lynch syndrome type II, tumors of other organs (endometrium, stomach, skin, and urinary tract, in particular) are also present in a given family. However, this is questioned by some authors who did not find evidence to justify this subdivision [66]. A representative pedigree of an Italian family with HNPCC (Lynch syndrome type II) is illustrated in Figure 1.

Due to the absence of morphological markers and the sophisticated technology required for molecular analysis, diagnosis of HNPCC is essentially based on clinical grounds. A panel of experts recently suggested a few "minimum criteria," the so-called "Amsterdam criteria," which should be met for a proper definition of HNPCC [107] (Table I). These criteria continue to provoke discussion and controversy. Some authors find them too "lax," others too "strict," and others much more suitable for type I than for type II Lynch syndrome [62]. However, despite some obvious limitations, the "Amsterdam criteria" are of great help in clinical practice, especially because they provide a basis of consistency in analyzing families with HNPCC or suspected HNPCC, and this is particularly valuable in collaborative investigations.

Modifications of the "Amsterdam criteria" have recently been proposed [5,87] which especially take into account the small size of many families and the frequent occurrence of cancer in other organs, two factors not considered in the Amsterdam criteria (Table II).

FREQUENCY OF HNPCC

HNPCC has been described in different racial groups (Caucasians, African Americans, Japanese, Filipinos, and Native Americans) [55,59,96]. However, in spite of numerous studies, the real prevalence of Lynch syndrome is still uncertain. Mecklin and Mecklin et al. [65,68] investigated the familial occurrence of cancer in a population consisting of all CRC cases diagnosed in a province of central Finland with approximately 250,000 residents during the period 1970–1979. When the only criterion was the presence of at least 3 cases of CRC among first-degree relatives, the authors found 13 HNPCC families; by applying the Amsterdam criteria, 11 families satisfied the minimum requisites. These findings provided a frequency of HNPCC on the order of 3.8% of all registered malignancies of the large bowel. Surprisingly, these observations were not confirmed by a subsequent prospective Finnish study, in which the proportion of HNPCC ranged between 1–2% of all CRCs [69]. Using the data of a population-based cancer registry, Ponz de Leon et al. [88] estimated the frequency of HNPCC in northern Italy to be on the order of 3.4–4.5% of all registered CRCs. Similar estimates (3.4%) were reported by Westlake et al. [113] in southern Alberta. Kee and Collins [34] detected only 13 families with a strong clinical suspicion of HNPCC out of 1,241 cases of CRC diagnosed in Northern Ireland, corresponding to an overall frequency of Lynch syndrome of 1–2% of all neoplasms of the large bowel.

In conclusion, the frequency of HNPCC seems to range between 1–5% of all CRCs [69,85]. Variations in the estimate might be due to ethnic differences, but it is perhaps more likely that these depend on different clinical criteria used for definition of the syndrome. In addition, incomplete data collection, lack of verification of cancer diagnosis, and poor collaboration of relatives might further contribute to under- or overestimation of HNPCC in some of these studies. Finally, since "single cases," i.e., those due to new mutations, escape clinical detection, it follows that the real frequency of the disease presumably remains underestimated. Detection of these single cases might represent one of the major objectives of molecular genetics in the years to come.

TUMOR SPECTRUM

In about 50–70% of HNPCC patients, cancer develops at an early age (before 50), an observation which is at variance with the occurrence of sporadic colorectal tumors. In a large American HNPCC series, neoplasms of the large bowel represented 55% of all cancers and endometrial carcinomas 14%; gastric and skin tumors were also slightly more frequent than in the general population; multiple tumors of the large bowel occurred in approximately 20% of patients [19]. A similar tumor spectrum was reported in various European series [67,106,108]. In a large Italian kindred, Cristofaro et al. [12] found an excess of gastric cancer and chronic gastritis of the antrum. The frequency of specific cancer types was recently studied in 23 large kindreds reported by Watson and Lynch [112]. By comparing the observed number of cancers to the expected rates from tumor registries, the authors showed that tumors of the stomach, small intestine, hepatobiliary system, urinary tract, uterus, and ovary all occurred significantly more often in HNPCC family members than in the general population.

The rare Muir-Torre syndrome (MTS), which is characterized by the appearance of cutaneous sebaceous tumors, shares several manifestations with HNPCC, namely predisposition to CRC and other neoplasms [56]. At least one subtype of MTS is an allelic variant of HNPCC, as demonstrated by cosegregation of *hMSH2* mutations in two large pedigrees [38a]. More recently, a subtype of Turcot syndrome (TS), characterized by the occurrence of glioblastomas, has been shown to share common molecular defects with HNPCC [26]. The germline molecular lesion differentiates this con-

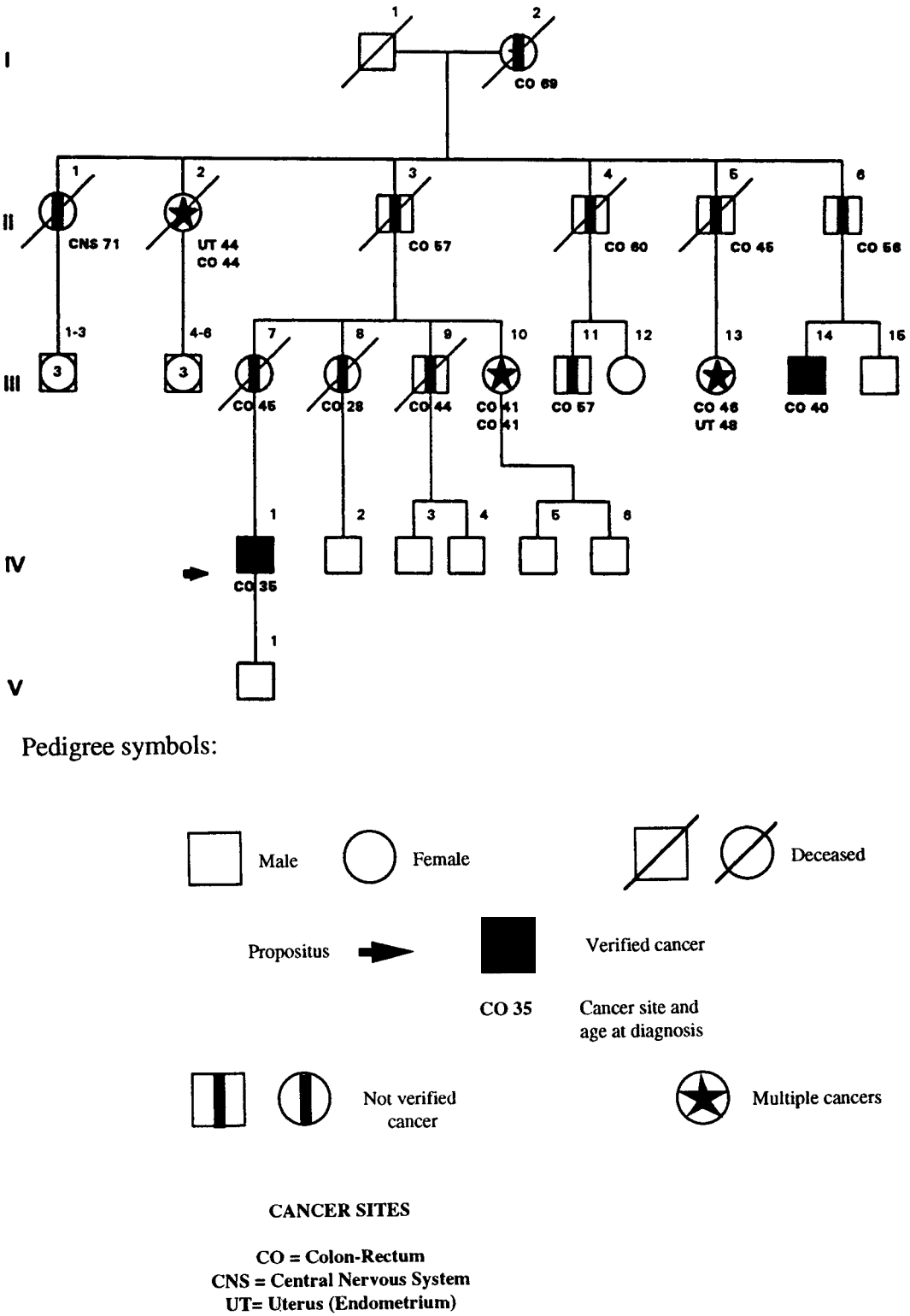

Fig. 1. Representative pedigree of a Lynch syndrome type II family. Note: a) the frequency of multiple tumors; b) the association of colonic-endometrial cancer in 2 patients; and c) the presence of colorectal carcinoma in four generations with a clear tendency to an earlier appearance in successive generations (I-2, age 69; II-3, age 57; III-7, age 45; IV-1, age 35).

TABLE I. The Minimum Criteria for Clinical Definition of HNPCC (Amsterdam Criteria) *

A)	At least three relatives should have histologically verified cancer of the large bowel; one of them should be first-degree relative to the other two; FAP should be excluded.
B)	At least two successive generations should be affected.
C)	In one of the relatives, colorectal cancer should be diagnosed before age 50 years.

*Reference no. [107]

dition with respect to the TS variant associated with medulloblastoma, which is caused by mutations in the *APC* gene.

Thus, the tumor spectrum of Lynch syndrome does not seem to be limited to a few neoplasms, as initially suggested. Indeed, careful observation of these families over the last 20 years has shown frequent occurrence of many other cancer types, suggesting a marked phenotypic variability of the syndrome. The expanding neoplastic spectrum further complicates the clinical management and surveillance of high-risk individuals in these families.

PATHOLOGY

Lynch syndrome is usually designated as "nonpolyposis" CRC. This definition underlines that HNPCC and FAP are different nosologic entities, an issue recently clarified by molecular biology, but does not imply the absence of colorectal adenomas in HNPCC. As a matter of fact, there are studies indicating that common adenomatous polyps are more frequent in HNPCC than in the general population, although other investigators have failed to show appreciable differences [41,60,66]. In addition, Jass and Stewart [31] found that adenomas tend to be larger and more often exhibit a villous pattern in HNPCC patients and at-risk family members. Taken together, these findings indicate that adenomas represent precancerous lesions in Lynch syndrome as well as in sporadic CRC.

There is no single histomorphological parameter which differentiates colorectal carcinomas occurring in Lynch syndrome from their sporadic counterpart, though according to some authors, hereditary colorectal tumors frequently show morphological aspects indicative of a more aggressive clinical behavior, including: 1) mucinous histology, which, according to some studies, is present in 30–40% of HNPCC tumors as opposed to 10–15% of sporadic CRCs [66]; 2) poor degree of differentiation; 3) infiltrating pattern of growth; and 4) aspects of neuroendocrine differentiation [60]. At variance with these observations, other studies have shown histological aspects usually associated with a more favorable clinical outcome, such as the presence of a dense lymphocytic infiltrate, and a definite excess of diploid tumor cells [20,39]. More recent studies have failed to show appreciable differences in proliferative activity, microvessel distribution, or pattern of oncogene expression between sporadic and hereditary CRCs [53].

TABLE II. Recently Proposed Modifications of Amsterdam Criteria for Clinical Definition of HNPCC *

A)	Very small families, which cannot be further expanded, can be considered as HNPCC even if only two colorectal cancers are found (of course, in the presence of the other criteria).
B)	In families with two first-degree relatives affected by colorectal cancer, the presence of a third relative with an early-onset unusual neoplasm or endometrial cancer is sufficient to consider the family as HNPCC.
C)	Age 55 (instead of 50) is suggested as upper limit for definition of early-onset cancer.
D)	Neoplasms are considered as "verified" when histological reports, clinical charts, or death certificates are available.

*Reference nos. [5,87].

FORMAL GENETICS

Familial aggregation of CRC could be the result of either hereditary or nonhereditary factors, such as environmental determinants shared by relatives, e.g., diet, lifestyle, or exposure to carcinogens. Chance clustering due to high prevalence of the disease in the general population further complicates the issue. The first convincing evidence that a genetic factor underlies at least a fraction of familial cases of CRC came from segregation studies. The results of an investigation of 11 extended HNPCC families favored an autosomal-dominant mode of inheritance [4]. Burt et al. [10] and Cannon-Albright et al. [11] analyzed the pattern of transmission of colorectal tumors (both carcinomas and adenomatous polyps, detected at endoscopy) in 34 large kindreds characterized by a striking aggregation of cancer in several generations. The results of this study confirmed that autosomal-dominant transmission of a single gene could explain susceptibility to both cancer and polyps. Similar results were obtained by Ponz de Leon et al. [89] and subsequently by Scapoli et al. [97], who in a study of 28 Italian HNPCC families estimated the frequency of the predisposing gene to be 0.0044 with a lifetime penetrance of 0.72 in heterozygotes, providing an incidence estimate of 5–6/1,000.

More recently, following a whole-genome search with 345 microsatellite markers, Peltomäki et al. [83] reported linkage between HNPCC and markers from chromosome 2p in two large kindreds segregating both CRC and other neoplasms. In this analysis not only CRC but also endometrial carcinoma was considered as a definite manifestation of the disease. However, analysis of 14 additional smaller kindreds provided evidence of genetic heterogeneity [1]. This result was confirmed by Lindblom et al. [47], who described an HNPCC family displaying linkage to markers from chromosome 3p. In another survey conducted on 13 large HNPCC kindreds, close linkage to chromosome 2p and 3p loci was found in 6 (46.1%) and 4 (30.7%) families, respectively. One family was unlinked to both loci. For the remaining two families the analysis was inconclusive. This study suggests that in 75–80% of families, HNPCC is linked to either chromosome 2p or 3p [78].

MICROSATELLITE INSTABILITY: THE RER$^+$ PHENOTYPE

At the time of the initial linkage studies, a surprising genetic alteration was identified in tumors from patients affected with HNPCC. Shifts in the electropho-

retic mobility of microsatellite markers were present in tumor DNA in comparison with normal (peripheral blood or unaffected colonic mucosa) DNA from the same patient [1,30,104]. For any given tumor, mobility shifts due to deletion or expansion of one or more repeat units were observed at several loci spaced all over the genome, occurring mostly within dinucleotide repeats, usually $(CA)_n$ repeats, but also in trinucleotide repeats. Although displaying a mutation rate higher than other genomic loci, microsatellites are normally stably inherited through generations and mutate appreciably only on an evolutionary scale. Therefore, the mobility shifts were interpreted as the end result of genomic instability leading to replication errors (RER). Nearly all tumors from HNPCC patients displayed the RER^+ phenotype (i.e., instability of at least two loci), whereas only 12–16% of sporadic CRCs did so [1,2,30,104]. Sporadic RER^+ CRCs were also similar to RER^+ HNPCC in many clinicopathological variables: prevalent localization in the right colon, normal or near-normal ploidy, less frequent presence of metastases at diagnosis, and overall better prognosis than RER^- CRCs [1,104]. The main conclusion was that mutations in HNPCC genes are responsible for genomic instability through a generalized defect in replication/repair processes [1].

Following the description of microsatellite instability in hereditary and sporadic CRCs, a host of reports has been published describing this genetic alteration in several other human cancers, including breast [115] and lung carcinomas [71,101], soft tissue sarcomas [114], and meningiomas [92]. Remarkably high frequencies of genomic instability have been noted in some cancers, such as pancreatic (67%) [27], gastric (18–31%) [73,93], and endometrial (17–23%) [9,95], which are main components of the HNPCC spectrum.

However, the observation of the RER^+ phenotype in such a wide range of cancers points to the need for establishing standardized procedures to assess microsatellite instability, both in terms of the number and type of loci investigated, and of the minimal number of altered loci necessary to define a tumor as RER^+.

THE MISMATCH REPAIR SYSTEM

The first evidence linking microsatellite instability to a defect in DNA repair was obtained in microorganisms. A specialized DNA repair system, the so-called mismatch repair system, has been described both in *E. coli* and *S. cerevisiae*. Deletions and expansions, occurring at a high rate during microsatellite replication, and due to slippage of the leading or lagging DNA strand, are normally repaired by this system [40,45]. In mutant strains, length instability of $(CA)_n$ repeats increases approximately 100–700-fold compared to wild-type cells. On the other hand, mutations in the 3′–5′ exonuclease (proofreading) activity of yeast DNA polymerase δ have only a modest effect on repeat instability, with a 5–10-fold increase [103].

In *E. coli*, the best-defined mismatch repair pathway is the methyl-directed system. This system handles base-base mismatches, short insertions/deletions, and recombination-derived heteroduplexes. Repair by this pathway requires 10 biochemical activities and proceeds in three steps: initiation, excision, and resynthesis [74]. In the first step the mismatch is detected and a single-strand cut is made on the newly synthesized DNA strand containing the mutation; the new strand is identified by virtue of the lack of adenine methylation at d(GATC) sites. Then, a tract of about 1–2 kb containing the mismatch is excised, and, lastly, resynthesis occurs. Initiation is mediated by the products of the *mutSLH* genes. The protein MutS recognizes and binds to DNA containing a mismatch. Following interaction with MutL, which likely acts as an interface between MutS and MutH, a latent single-strand endonuclease activity of the latter is activated, and incision of the unmethylated strand near a d(GATC) site takes place. Other enzymes required for subsequent steps are DNA helicase II (gene product of *mutU*), single-strand exonucleases (exo I, exo VII, RecJ) which remove a fragment of DNA containing the mismatched region, DNA polymerase III holoenzyme, single-strand binding protein (SSB), and, finally, DNA ligase, which seals the DNA nick (Fig. 2) [74,75]. Eukaryotic cells follow a very similar biochemical pathway, characterized by strand-specificity, bidirectional excision capability, and employment of the replicative DNA polymerase α [16,29]. The molecular determinants of strand-specificity in eukaryotic cells, which lack d(GATC) modification, are not known; some evidence points to cytosine hemimethylation at CpG sites [74].

HUMAN MISMATCH REPAIR GENES AND HNPCC

Based upon data obtained in bacteria and yeast that defects in DNA mismatch repair are associated with genomic instability, it was readily predicted that HNPCC, with tumors characterized by the RER^+ phenomenon, might be caused by germline mutations in mismatch repair genes [103].

The first human mismatch gene to be cloned was *hMSH2,* the homolog of yeast *MSH2* and *E. coli mutS*. Cloning took advantage of the high degree of evolutionary conservation of the gene and was performed using degenerate polymerase chain reaction (PCR) [18,43]. The gene was shown to map on chromosome 2p16, and germline mutations cosegregating with the disease were identified in the two original large kindreds with established linkage of HNPCC to chromosome 2p [43]. A recent multiassay survey of *hMSH2* mutations in HNPCC kindreds showed that at least 40% of cases might be associated with germline mutations of this gene. Moreover, 9 of 10 identified mutations resulted in truncated proteins and therefore could be detected by a coupled in vitro transcription-translation assay [49].

The second human mismatch gene, *hMLH1,* was cloned by degenerate PCR [8] and by screening a database of cDNA sequences (expressed sequence tags, EST) [80]. *hMLH1,* which displays a striking homology to yeast *MLH1* and *E. coli MutL,* was mapped to chromosome 3p21.3. As anticipated, germline mutations in *hMLH1* were identified in affected members of HNPCC kindreds linked to chromosome 3p [8,80]. Most of the mutations appeared to alter the size of the hMLH1 protein [49a,80]. Interestingly, most Finnish HNPCC fam-

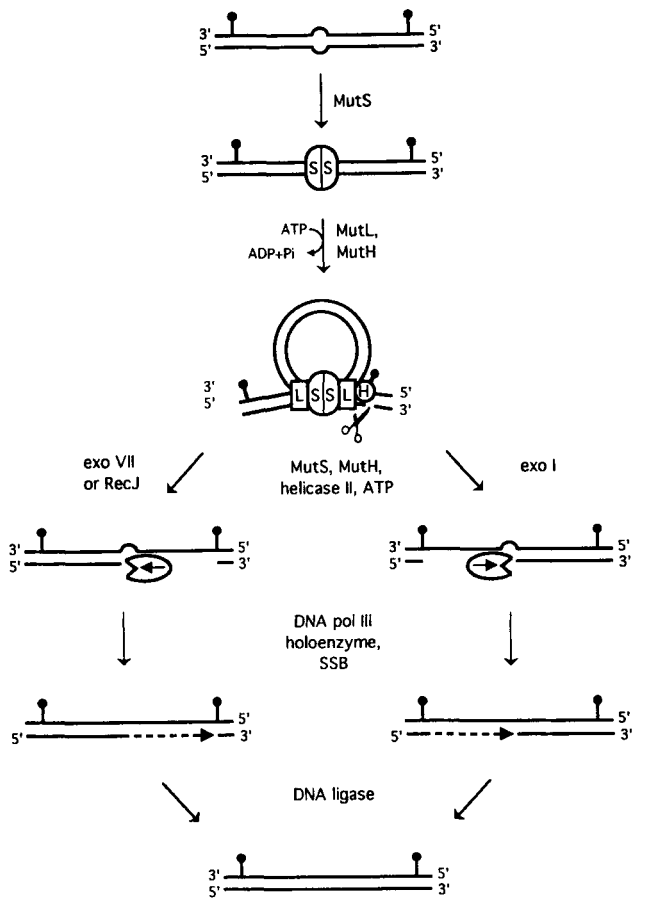

Fig. 2. The *E. coli* mismatch repair system. Mismatch is detected by the protein MutS (here shown to bind DNA as a dimer), which generates α-shaped DNA loops. Loops are probably necessary to bring into close proximity the mismatch and the nearest hemimethylated d(GATC) site (indicated by ↟). Following interaction with MutL, MutH makes a single-strand cut on the new (unmethylated) DNA strand. After DNA unwinding by DNA helicase II, a DNA tract of about 1–2 kb containing the mismatch is excised by single-strand exonucleases (exo I, exo VII, or RecJ). Since incision can occur on either side of the mismatch, excision can proceed in either direction with respect to the mismatch. Typically, resynthesis is mediated by the replicative DNA polymerase III holoenzyme and single-strand binding protein (SSB). Lastly, DNA ligase seals the DNA nick (from [74,75], modified).

ilies share an ancestral chromosome 3 haplotype, indicative of a founder effect [79].

During the above-mentioned EST screening, two additional *mutL*-related genes were identified and designated *hPMS1* and *hPMS2*, because of homology to the yeast *mutL* homologue *PMS1*. *hPMS1* and *hPMS2* were each found mutated in 2 patients affected with HNPCC [77].

Mutations of another *mutS* homologue, *GTBP/P160*, have been detected in hypermutable CRC cell lines [80a]. Its protein product is found in heterodimers containing the product of the *hMSH2* gene, and its role in DNA repair appears to be limited to the recognition of G/T mispairs and 1-bp insertions/deletions [14b,79a,80a].

Additional *mutS* and *mutL* homologues have been identified [21,29a], but their role in human disease has not yet been investigated. However, the involvement of additional genes in the pathogenesis of HNPCC is likely. In fact, preliminary results of mutational analysis in Asiatic [27a] and Mediterranean populations [Genuardi et al., unpublished] indicate that at least *hMSH2* may be less frequently involved than expected on the basis of the results of previous linkage studies.

Finally, in RER$^+$ CRC cell lines negative for mutations in mismatch repair genes, variants of DNA polymerase δ of unknown functional significance have been identified, which are characterized by differences in the 3′–5′ exonuclease domain [14]. The similarity with the above-described yeast mutants is striking and indicates that mutations in the proofreading activity of replicative polymerase δ might cause microsatellite instability.

The main characteristics of human mismatch repair genes involved in cancer are shown in Table III.

The identification and sequencing of the mismatch repair genes involved in HNPCC paved the way to the molecular diagnosis of this disorder. In the absence of a simple functional test capable of measuring mismatch repair proficiency, molecular diagnosis of HNPCC is based on direct mutational analysis of the genes on normal (peripheral blood) DNA and/or RNA [48,49a].

The genetic evidence linking mismatch repair genes to HNPCC and the RER$^+$ phenotype has been further substantiated both by biochemical experiments which unequivocally show that RER$^+$ cells have a defective mismatch repair system [81,105], and by the finding of >100-fold increases in the mutation rate at the *HPRT* reporter locus in RER$^+$ cell lines established from CRCs [5a,15a].

However, an important question yet to be answered is whether the mismatch repair gene defects act as recessive or dominant phenotypes at the cellular level, i.e., whether or not both alleles of a mismatch repair gene need to be inactivated for the cell to display a mutator phenotype with ensuing microsatellite instability. Mice carrying two copies of an inactive *hMSH2* allele develop lymphomas at an early age, whereas tumors were not observed in heterozygotes [14b]. The lack of RER$^+$ phenotype in normal cells from HNPCC-affected individuals confirms that one functional copy of a mismatch repair gene is sufficient to guarantee an active repair system. Accordingly, a lymphoblastoid cell line from an HNPCC patient was shown to be repair-proficient on mismatched heteroduplex substrates [81]. However, in another study, microsatellite instability was observed in a fraction of cells from carriers of known *hMLH1* and *hPMS2* mutations [81a]. This apparent discrepancy could be explained by dominant negative effects of specific mutations.

Repair-deficient cell lines and HNPCC tumors have been described as carrying both a germline and a somatic alteration in mismatch repair genes [43,48,77]. This situation is clearly reminiscent of Knudson's two-hit hypothesis for tumor-suppressor genes [38]. Most often, cells carrying a germline mutation in a tumor-suppressor gene acquire the second inactivating mutation as a result of a chromosomal deletion, and this is revealed as loss of heterozygosity (LOH) at the corresponding locus [84]. Actually, LOH at the *hMLH1* locus

TABLE III. Human Mismatch Repair Genes Involved in Cancer

Gene	Homologue		Function	cDNA (bp)	Protein (amino acids)	Chromosome
	E. coli	*S. cerevisiae*				
hMSH2	*mutS*	*MSH2*	Mismatch binding	3,111	934	2p16
GTBP	*mutS*	?	Mismatch binding	~4,200	?	2p16
hMLH1	*mutL*	*MLH1*	Complex formation	2,484	756	3p21.3–23
hPMS1	*mutL*	*PMS1*	Complex formation	3,063	932	2q31–33
hPMS2	*mutL*	*PMS1*	Complex formation	2,771	832	7p22

occurs frequently in tumors from *hMLH1*-associated HNPCC families, and targets the wild-type allele [27].

As for other tumor suppressors, both inactivating mutations can be somatic, as seen for RER$^+$ CRCs lacking any family history [49a]. This brings up the issue of whether screening for RER$^+$ tumors might help in recognition of HNPCC. It is certainly possible that a fraction of cancers displaying a bona fide RER$^+$ phenotype, particularly if belonging to the HNPCC tumor spectrum, may actually derive from germline mutations of HNPCC genes. In fact, not only for CRC, but also for endometrial cancer, there is a marked difference in the frequency of microsatellite instability between sporadic and HNPCC cases (17% vs. 75%, respectively) [95]. However, in a recent survey, only 1 of 10 RER$^+$ "sporadic" CRCs carried a germline mutation in a mismatch repair gene [49a]. The obvious consequence is that analysis of microsatellite instability is not applicable as a screening test to detect HNPCC, since only a minor fraction of cases would turn out to be related to a constitutional predisposition.

ONCOGENESIS BY DEFECTIVE MISMATCH REPAIR

The discovery that a defective repair system is involved in the pathogenesis of HNPCC and at least a fraction of sporadic CRCs sheds new light on the role that an increased mutation rate plays in tumor induction and progression. In the Western world, the incidence of CRC, as well as of other cancer types, increases with age and is proportional to the third/seventh power of elapsed time [72]. This suggests that the elapsed time reflects the need for 4–8 independent genetic events to accumulate in a single cancer cell (multistep carcinogenesis). Molecular analysis of CRC has confirmed that five or more genetic alterations of oncogenes and tumor suppressor genes are required for onset of carcinoma, and fewer for adenoma [6,17,110]. However, it is unlikely that several independent mutations accumulate in a single cell at the spontaneous mutation rate. Therefore, it is assumed that in tumor cells the mutation rate increases during progression (Loeb's mutator hypothesis) [50,51]. Moreover, the mutation rate is critically limiting in the early steps of tumorigenesis, when the number of premalignant target cells is small [6]. In fact, microsatellite instability has been shown to occur at the stage of early adenomas and to persist throughout tumor progression [100]. Along those lines, it should be pointed out that according to some authors the incidence of adenomas in HNPCC, unlike FAP, appears to be low, although the progression of adenoma to carcinoma is significantly accelerated [31,61].

Mutations in mismatch repair genes would not cause a direct proliferative advantage; rather, they would facilitate the occurrence of mutations in target oncogenes and tumor suppressor genes [18,43,49a]. Therefore, it is expected that the same genes involved in the pathogenesis of sporadic CRC are also involved in HNPCC. The incidence of somatic mutations in *KRAS, P53,* and *APC* does not differ in HNPCC as compared to sporadic CRC [1]. However, the genetic mechanisms involved could be different. It is assumed that HNPCC cells are prone not merely to microsatellite instability but also to transitions and transversions [81]. It is intriguing that the most prevalent *KRAS* mutation in CRC (G→A) [7] could result from an unrepaired G-T mispair. G-T mispairs may also originate from deamination of 5-methylcytosine (G−m^{5}C→G-T), which has been shown to function in endogenous mutagenesis in the *P53* gene [94]. Similarly, several insertion/deletion mutations in *P53* and *APC* occur at sites flanked by repeated sequences [23,32]. Interestingly, CRC from a patient carrying an *hMSH2* germline mutation has been found to contain six and four independent mutations in the *APC* and *P53* genes, respectively [42]. Most mutations were frameshifts and G→A transitions, as expected for a mismatch repair defect. Moreover, inactivating mutations of the type II TGF-β receptor frequently occur in RER$^+$ CRC cell lines, and are localized at short stretches of mono- and dinucleotide repeats [63a]. Although LOH at several loci is a common event in sporadic CRC, it has been reported that the RER$^+$ phenotype is inversely correlated with genome-wide LOH [1] and with LOH for markers on 5q (*APC*), 17p (*P53*), and 18q (*DCC*) [104]. This confirms the finding that HNPCC tumors are often diploid or near-diploid, and suggests that tumorigenesis mediated by a defective mismatch repair does not require gross chromosomal changes for the accumulation of genetic alterations [6]. However, since at least the *hMSH2* and *hPMS2* genes are involved in the prevention of inappropriate genetic recombination in mice [4a,14a], it is possible that additional mutational mechanisms yet to be demonstrated are active in cells carrying nonfunctional mismatch repair genes.

The continuous mutational load suffered by mismatch repair-deficient tumor cells might also cause them to have a relatively low fitness and, perhaps, to be more immunogenic. This could explain the relatively better prognosis of both HNPCC and sporadic RER$^+$ CRCs [6].

An issue which awaits further investigation is the identification of molecular and physiopathological determinants of the HNPCC tumor spectrum. Mismatch repair genes are presumably housekeeping genes [43]; however, it is not clear why tumor development is restricted to some organs and why the colon is a preferential target. One possibility is that colonic mucosa is exposed to a higher mutational burden for a longer time than other tissues, e.g., carcinogens contained in food, toxic metabolites, and bacterial products. This consideration might also apply to bladder transitional epithelium. Moreover, colonic mucosa has a very high proliferative rate, and it is conceivable that rapid proliferation might saturate and exhaust the mismatch repair machinery [40,98]. This might be also true for endometrial mucosa during regeneration.

TREATMENT AND FOLLOW-UP

The optimal treatment of a newly diagnosed CRC in HNPCC includes two main options: 1) subtotal colectomy with ileorectal anastomosis [19]; or 2) right (or left) hemicolectomy with careful endoscopic surveillance of the remaining large bowel. Although chemotherapy is usually not considered, it should be noted that mismatch repair-deficient cells have been shown to be tolerant to killing by alkylating agents [7a]. Owing to the high risk of endometrial cancer in these patients, prophylactic hysterectomy, at the time of surgery for CRC, has been proposed, or, as an alternative approach, biannual pelvic (or intravaginal) ultrasounds and annual endometrial aspiration biopsy. In addition, an individualized surveillance program should be designed on the basis of the cancer type observed in each family [61].

Although there is a general consensus that individuals at risk for cancer in HNPCC families should be followed at regular intervals, no standard protocol has been developed and accepted. The strategy proposed by the Colorectal Cancer Study Group of the University of Modena, Italy, for following high-risk subjects in HNPCC families, can be summarized as follows [5]:

1) Pancolonoscopy, by far the most important clinical investigation, should be recommended to all first-degree relatives of affected patients, usually starting at age 25 years and then repeated every 2–3 years.

2) Upper gastrointestinal endoscopy is suggested only for those families in which gastric carcinoma has occurred, especially with an early age of onset (<50 years).

3) Gynecological investigations (including lower abdominal ultrasounds) are suggested to all women at risk, though the real benefit of screening for endometrial and ovarian carcinoma remains questionable.

4) Other more specific clinical investigations (such as urography, cholangiography, or computed tomography) are usually recommended in selected cases.

5) Since the genetic basis of HNPCC does not exclude the possible role of environmental factors, the patient is advised to modify dietary habits (increasing the amounts of vegetables, fruit, and fiber, and reducing intake of meat and animal fat), to reach and maintain the ideal body weight, and to spend more time in physical activity.

At present, these suggestions are given to all at-risk individuals in HNPCC families (including, of course, patients who have undergone colorectal surgery).

PROSPECTS FOR GENETIC COUNSELING

Due to the pacebreaking course of discoveries in the molecular genetics of HNPCC, with only a few months from regional chromosome mapping to identification of the first mutant gene, counseling experience based on linkage analysis has been very limited, and it is likely that the major impact of molecular advances will be on the refinement of predictive counseling for at-risk patients.

In HNPCC kindreds, finding a germline mutation in the index patient is a prerequisite for the identification of other heterozygotes at risk for CRC. These individuals should be offered extensive counseling, covering both risk estimation and information on the type and efficiency of measures available for prevention and early diagnosis. On the other hand, individuals recognized as not being carriers of predisposing mutations should be excluded from monitoring programs. It will also be possible to identify "background" CRCs, i.e., phenocopies, which may occur in HNPCC families just as in the general population, as already shown for breast cancer in pedigrees segregating *BRCA1* mutations [36]. Consequently, surveillance and prevention efforts will be focused on mutation carriers, allowing a more rational application and a more accurate interpretation of the results, in terms of psychological consequences, side effects, and reduction of cancer morbidity and mortality.

Undoubtedly, presymptomatic genetic testing will not solve all old questions, and new ones will surface until the molecular pathophysiology of mismatch repair is fully unravelled. Protein changes of uncertain functional significance have already been identified [18,48], and this list will presumably grow, considering that at least four genes are implicated in HNPCC. In principle, functional assays are already available for the purpose of discriminating between harmless polymorphisms and pathogenic mutations [81], but the current methodology is not suitable for large-scale surveys, and its application will necessarily be restricted to a minority of centers. The development of diagnostic functional tests would also help to define the degree of cancer susceptibility conferred by different mutations. Data from hereditary retinoblastoma [52], von Hippel-Lindau disease [13], and familial adenomatous polyposis [90] indicate that different tumor suppressor mutations may not be equally penetrant. In fact, the finding of several unaffected mutation carriers, who were relatives of patients who developed CRC at an early age [49], indicates that the penetrance of HNPCC may be lower than previously inferred from segregation analysis.

Epidemiological evaluation of genotype-phenotype correlations will lead to a better understanding of the wide variation in sites of neoplastic development, and, specifically, of the intriguing difference between type I and type II HNPCC. This situation is not peculiar to

HNPCC, since other cancer-predisposing genes, such as *BRCA1* and *P53*, show marked interfamilial phenotypic variability in tumor spectrum [15,25,63,91,102]. It is evident that such eagerly awaited achievements are necessary for tailoring genetic counseling to individual situations, with the aim of providing more accurate presentations of risks and monitoring or preventive options.

Although population screening is presently not applicable, this possibility should be discussed before a quick and reliable diagnostic test is available. Issues of debate concern both the target population and the care provider. For patients: who should be offered the test and how is it accessed? Who has the moral right to allow testing of minors? How is inappropriate handling of results by employers and health insurance companies avoided? For providers of diagnostic services: who should formulate technical and ethical standards, and who should survey them?

Although dietary habits have been implicated in the pathogenesis of common sporadic colorectal carcinomas, the existence of confounding factors often hampers the interpretation of epidemiological surveys. Paradoxically, a substantial contribution to the definition of risk magnitudes associated with specific dietary components or other environmental agents might be provided by the study of inherited tumors caused by germline alterations in DNA repair mechanisms. Development of oncogenic mutations in homozygous mismatch repair-deficient cells could be accelerated relative to normal cells if the former were more sensitive to the genotoxic effects of some mismatch-inducing carcinogens. In fact, a significantly accelerated rate of tumor development following exposure to ionizing radiation has been observed in knockout mice lacking one or both *P53* alleles [35], presumably as a consequence of inefficient repair in the G1 phase of the cell cycle. Ultimately, the merging of molecular and epidemiological expertise applied to HNPCC could define the basis of primary prevention, not only of hereditary CRC, but also of the more prevalent sporadic form.

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REFERENCES

1. Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin J-P, Järvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, de la Chapelle A (1993): Clues to the pathogenesis of familial colorectal cancer. Science 260:812–816.
2. Aaltonen LA, Peltomäki P, Mecklin J-P, Järvinen H, Jass JR, Green JS, Lynch HT, Watson P, Tallqvist G, Juhola M, Sistonen P, Hamilton SR, Kinzler KW, Vogelstein B, de la Chapelle A (1994): Replication errors in benign and malignant tumors from hereditary non-polyposis colorectal cancer patients. Cancer Res 54:1645–1648.
3. Allum WH, Slaney G, McConkey CC, Powell J (1994): Cancer of the colon and rectum in the West Midlands, 1957–1981. Br J Surg 81:1060–1063.
4. Bailey-Wilson JE, Elston RC, Schuelke GS, Kimberling W, Albano W, Lynch JF, Lynch HT (1986): Segregation analysis of hereditary nonpolyposis colorectal cancer. Genet Epidemiol 3:27–38.

4a. Baker SM, Bronner E, Zhang L, Plug AW, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM (1995): Male mice defective in the mismatch repair gene *PMS2* exhibit abnormal chromosome synapsis in meiosis. Cell 82:309–319.

5. Benatti P, Sassatelli R, Roncucci L, Pedroni M, Fante R, Di Gregorio C, Losi L, Gelmini R, Ponz de Leon M (1993): Tumour spectrum in hereditary non-polyposis colorectal cancer and in families with "suspected HNPCC." A population-based study in northern Italy. Int J Cancer 54:371–377.

5a. Bhattacharyya NP, Skandalis A, Ganesh A, Groden J, Meuth M (1994): Mutator phenotypes in human colorectal carcinoma cell lines. Proc Natl Acad Sci USA 91:6319–6323.

6. Bodmer W, Bishop T, Karran P (1994): Genetic steps in colorectal cancer. Nature Genet 6:217–219.
7. Bos JL (1989): *ras* oncogenes in human cancer: A review. Cancer Res 49:4682–4689.

7a. Branch P, Hampson R, Karran P (1995): DNA mismatch binding defects, DNA damage tolerance, and mutator phenotypes in human colorectal carcinoma cell lines. Cancer Res 55:2304–2309.

8. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjøld M, Fishel R, Kolodner R, Liskay RM (1994): Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. Nature 368:258–261.
9. Burks RT, Kessis TD, Cho KR, Hedrick L (1994): Microsatellite instability in endometrial carcinoma. Oncogene 9:1163–1166.
10. Burt RW, Bishop TD, Cannon LA, Dowdle MA, Lee RG, Skolnick MH (1985): Dominant inheritance of adenomatous colonic polyps and colorectal cancer. N Engl J Med 312:1540–1544.
11. Cannon-Albright L, Skolnick MH, Bishop TD, Lee RG, Burt RW (1988): Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. N Engl J Med 319:533–537.
12. Cristofaro G, Lynch HT, Caruso ML, Attolini A, Matteo GI, Giorgio P, Senatore S, Argentieri A, Sbano E, Fusaro R, Giorgio I (1987): New phenotypic aspects in a family with Lynch syndrome II. Cancer 60:51–58.
13. Crossey PA, Richards FM, Foster K, Green JS, Prowse A, Latif F, Lerman MI, Zbar B, Affara NA, Ferguson-Smith MA, Maher ER (1994): Identification of intragenic mutations in the von Hippel-Lindau disease tumor suppressor gene and correlation with disease phenotype. Hum Mol Genet 3:1303–1308.
14. Da Costa LT, Liu B, El-Diery WS, Hamilton SR, Kinzler KW, Vogelstein B, Markowitz S, Willson JKV, de la Chapelle A, Downey KM, So AG (1995): Polymerase variants in RER$^+$ colorectal tumours. Nature Genet 9:10–11.

14a. De Wind N, Dekker M, Berns A, Radman M, te Riele H (1995): Inactivation of the mouse *Msh2* gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell 82:321–330.

14b. Drummon JT, Li G-M, Longley MJ, Modrich P (1995): Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science 268:1909–1912.

15. Easton DF, Bishop DT, Ford D, Crockford GP, Breast Cancer Linkage Consortium (1993): Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. Am J Hum Genet 52:678–701.

15a. Eshleman JR, Lang EZ, Bowerfind GK, Parsons R, Vogelstein B, Wilson JK, Veigl ML, Sedwick WD, Markowitz SD (1995): Increased mutation rate at the *hprt* locus accompanies microsatellite instability in colon cancer. Oncogene 10:33–37.

16. Fang W-H, Modrich P (1993): Human strand-specific mismatch repair occurs by a bidirectional mechanism similar to that of the bacterial reaction. J Biol Chem 268:11838–11844.

17. Fearson ER, Vogelstein B (1990): A genetic model for colorectal tumorigenesis. Cell 61:759–767.
18. Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R (1993): The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell 75:1027–1038.
19. Fitzgibbons RJ, Lynch HT, Stanislav GV, Watson PA, Lanspa SJ, Markus SN, Smyrk T, Kriegler MD, Lynch JF (1987): Recognition and treatment of patients with hereditary non-polyposis colorectal cancer. Ann Surg 206:289–294.
20. Frei JV (1992): Hereditary nonpolyposis colorectal cancer: Diploid malignancies with prolonged survival. Cancer 69:1108–1111.
21. Fujii H, Shimada T (1989): Isolation and characterization of cDNA clones derived from the divergently transcribed gene in the region upstream from the human dihydrofolate reductase gene. J Biol Chem 264:10057–10064.
22. Gray D, Lee H, Schlinkert R, Beart RW Jr (1994): Adequacy of lymphadenectomy in laparoscopic-assisted colectomy for colorectal cancer: A preliminary report. J Surg Oncol 57:8–10.
23. Groden J, Gelbert L, Thliveris A, Nelson L, Robertson M, Joslyn G, Samowitz W, Spirio L, Carlson M, Burt R, Leppert M, White R (1993): Mutational analysis of patients with adenomatous polyposis: Identical inactivating mutations in unrelated individuals. Am J Hum Genet 52:263–272.
24. Haenszel W (1982): Migrant studies. In Schottenfeld D, Fraumeni JF (eds): "Cancer Epidemiology and Prevention." Philadelphia: W.B. Saunders, pp 194–207.
25. Hall JM, Lee MK, Mewman B, Morrow J, Anderson L, Huey B, King M-C (1990): Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250:1684–1698.
26. Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, Krush AJ, Berk T, Cohen Z, Tetu B, Burger PC, Wood PA, Taqi F, Booker SV, Petersen GM, Offerhaus GJA, Tersmette AC, Giardiello FM, Vogelstein B, Kinzler KW (1995): The molecular basis of Turcot's syndrome. N Engl J Med 332:839–847.
27. Han H-J, Yanagisawa A, Kato Y, Park J-G, Nakamura Y (1993): Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res 53:5087–5089.

27a. Han H-J, Maruyama M, Baba S, Park J-G, Nakamura Y (1995): Genomic structure of human mismatch repair gene, hMLH1, and its mutation analysis in patients with hereditary non-polyposis colorectal cancer (HNPCC). Hum Mol Genet 4:237–242.

28. Hemminki A, Peltomäki P, Mecklin J-P, Järvinen H, Salovaara R, Lahti MN, de la Chapelle A, Aaltonen LA (1994): Loss of the wild type *MLH1* gene is a feature of hereditary nonpolyposis colorectal cancer. Nat Genet 8:405–410.
29. Holmes J Jr, Clark S, Modrich P (1990): Strand-specific mismatch correction in nuclear extracts of human and Drosophila melanogaster cell lines. Proc Natl Acad Sci USA 87:5837–5841.

29a. Horii A, Han H-J, Sasaki S, Shimada M, Nakamura Y (1994): Cloning, characterization and chromosomal assignment of the human genes homologous to yeast *PMS1*, a member of mismatch repair genes. Biochem Biophys Res Commun 204:1257–1264.

30. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M (1993): Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 363:558–561.
31. Jass JR, Stewart SM (1992): Evolution of hereditary non-polyposis colorectal cancer. Gut 33:783–786.
32. Jego N, Thomas G, Hamelin R (1993): Short direct repeats flanking deletions, and duplicating insertions in p53 gene in human cancers. Oncogene 8:209–213.
33. Joslyn G, Carlson M, Thliveris A, Albertsen H, Gelbert L, Samowitz W, Groden J, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R (1991): Identification of deletion mutations and three new genes at the familial polyposis locus. Cell 66:601–613.
34. Kee F, Collins BJ (1991): How prevalent is cancer family syndrome? Gut 32:509–512.
35. Kemp CJ, Wheldon T, Balmain A (1994): P53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. Nature Genet 8:66–69.
36. King M-C, Rowell S, Love S (1993): Inherited breast and ovarian cancer. What are the risks? What are the choices? JAMA 269: 1975–1980.
37. Kinzler KW, Nilbert MC, Su KL, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKechnie D, Finniear R, Markham A, Groffen J, Boguski MS, Altschul SF, Horii A, Ando H, Mioshi Y, Miki Y, Nishisho I, Nakamura Y (1991): Identification of FAP locus genes from chromosome 5q21. Science 253:661–665.
38. Knudson AG (1985): Hereditary cancer, oncogenes and antioncogenes. Cancer Res 45:1437–1443.

38a. Kolodner RD, Hall NR, Lipford J, Kane MF, Rao MRS, Morrison P, Wirth L, Finan PJ, Burn J, Chapman P, Earabino C, Merchant E, Bishop DT (1994): Structure of the human MSH2 locus and analysis of two Muir-Torre kindreds for msh2 mutations. Genomics 24:516–526.

39. Kouri M, Laasonen A, Mecklin J-P, Järvinen H, Franssila K, Pyrhönen S (1990): Diploid predominance in hereditary nonpolyposis colorectal carcinoma evaluated by flow cytometry. Cancer 65:1825–1829.
40. Kunkel TA (1993): Slippery DNA and diseases. Nature 365:207–208.
41. Lanspa SJ, Jenkins JX, Watson P, Smyrk TC, Cavalieri RJ, Lynch JF, Lynch HT (1992): Natural history of at-risk Lynch syndrome family members with respect to adenomas. Nebr Med J 77:310–313.
42. Lazar V, Grandjouan S, Bognel C, Couturier D, Rougier P, Bellet D, Bressac-de Paillerets B (1994): Accumulation of multiple mutations in tumour suppressor genes during colorectal tumorigenesis in HNPCC patients. Hum Mol Genet 3:2257–2260.
43. Leach FS, Nicolaides NC, Papadopulos N, Liu B, Jen J, Parsons R, Peltomäki P, Sistonen P, Aaltonen LA, Nyström-Lahti M, Guan X-Y, Zhang J, Meltzer PS, Yu J-W, Kao F-T, Chen DJ, Cerosaletti KM, Foumler REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin J-P, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch HT, Trent JM, de la Chapelle A, Kinzler KW, Vogelstein B (1993): Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1216–1225.
44. Leppert M, Dobbs M, Scambler P, O'Connell P, Nakamura Y, Stauffer D, Woodward S, Burt R, Hughes J, Gardner E, Lathrop M, Wasmuth J, Lalouel J-M, White R (1987): The gene for familial polyposis coli maps to the long arm of chromosome 5. Science 238:1411–1413.
45. Levinson G, Gutman GA (1987): High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. Nucleic Acids Res 15:5323–5338.
46. Lieberman D (1994): Screening/early detection model for colorectal cancer. Why screen? Cancer 74:2023–2027.
47. Lindblom A, Tannergard P, Werelius B, Nordenskjold M (1993): Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. Nature Genet 5:279–282.
48. Liu B, Parson RE, Hamilton SR, Petersen GM, Lynch HT, Watson P, Markowitz S, Willson JKV, Green J, de la Chapelle A, Kinzler KW, Vogelstein B (1994): *hMSH2* mutations in hereditary nonpolyposis colorectal cancer kindreds. Cancer Res 54:4590–4594.

49a. Liu B, Nicolaides NC, Markowitz S, Willson JKV, Parsons RE, Jen J, Papadopolous N, Peltomaki P, de la Chapelle A, Hamilton SR, Kinzler KW, Vogelstein B (1995): Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nature Genet 9:48–55.

49. Liu B, Farrington SM, Petersen GM, Hamilton SR, Parsons R, Papadopoulos N, Fujiwara T, Jen J, Kinzler KW, Wyllie AH, Vogelstein B, Dunlop MG (1995): Genetic instability in the majority of young patients with colorectal cancer. Nature Med 1:348–352.
50. Loeb LA (1991): Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 51:3075–3079.
51. Loeb LA (1994): Microsatellite instability: Marker of a mutator phenotype in cancer. Cancer Res 54:5059–5063.
52. Lohmann DR, Brandt B, Hopping W, Passarge E, Horsthemke B (1994): Distinct RB1 gene mutations with low penetrance in hereditary retinoblastoma. Hum Genet 94:349–354.

53. Losi L, Fante R, Di Gregorio C, Aisoni ML, Lanza G, Maestri I, Roncucci L, Pedroni M, Ponz de Leon M (1996): Biological characterizaton of hereditary nonpolyposis colorectal cancer: I. Nuclear ploidy, AgNOR count, microvessel distribution, oncogene expression, and grade-related parameters. Am J Clin Pathol (in press).

54. Lynch HT (1986): Frequency of hereditary nonpolyposis colorectal cancer. Gastroenterology 90:486–489.

55. Lynch HT, Krush AJ (1967): Heredity and adenocarcinoma of the colon. Gastroenterology 53:517–527.

56. Lynch HT, Krush AJ (1971): Cancer family G revisited: 1895–1970. Cancer 27:1505–1511.

57. Lynch HT, Drouhard TJ, Schuelke GS, Biscone KA, Lynch JF, Danes BS (1985): Hereditary non polyposis colorectal cancer in a Navajo Indian family. Cancer Genet Cytogenet 15:209–213.

58. Lynch HT, Fusaro RM, Roberts L, Voorhees GJ, Lynch JF (1985): Muir-Torre syndrome in several members of a family with a variant of the cancer family syndrome. Br J Dermatol 113:295–301.

59. Lynch HT, Lanspa S, Smyrk T, Boman B, Watson P, Lynch J (1991): Hereditary nonpolyposis colorectal cancer: Genetics, pathology, natural history, and cancer control. Cancer Genet Cytogenet 53:143–160.

60. Lynch HT, Smyrk T, Watson P, Lanspa SJ, Boman BM, Lynch PM, Lynch JF, Cavalieri J (1991): Hereditary colorectal cancer. Semin Oncol 18:337–366.

61. Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, Cavalieri RJ, Boland CR (1993): Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: An updated review. Gastroenterology 104:1535–1549.

62. Lynch PM (1994): Hereditary nonpolyposis colorectal carcinoma (HNPCC): Clinical application of molecular diagnostic testing. Ann Med 26:221–228.

63. Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH (1990): Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233–1238.

63a. Markowitz S, Wang J, Myeroff L, Parsons R, Sun LZ, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B, Brattain M, Willson JKW (1995): Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science 268:1336–1338.

64. McMichael AJ, McCall MG, Hartshorne JM, Woodings TL (1980): Patterns of gastrointestinal cancer in European migrants to Australia: The role of dietary changes. Int J Cancer 25:431–437.

65. Mecklin J-P (1987): Frequency of hereditary colorectal carcinoma. Gastroenterology 93:1021–1025.

66. Mecklin J-P, Jarvinen HJ (1986): Clinical features of colorectal carcinoma in cancer family syndrome. Dis Colon Rectum 29:160–164.

67. Mecklin J-P, Järvinen HJ (1991): Tumor spectrum in cancer family syndrome. Cancer 68:1109–1112.

68. Mecklin J-P, Ponz de Leon M (194): Epidemiology of HNPCC. Anticancer Res 14:1625–1630.

69. Mecklin J-P, Järvinen HJ, Peltokallio P (1986): Cancer family syndrome: Genetic analysis of 22 Finnish kindreds. Gastroenterology 90:328–333.

70. Mecklin J-P, Svendsen LB, Peltomäki P, Vasen HFA (1994): Hereditary nonpolyposis colorectal cancer. Scand J Gastroenterol 29:673–677.

71. Merlo A, Mabry M, Gabrielson E, Vollmer R, Baylin SB, Sidransky D (1994): Frequent microsatellite instability in primary small cell lung cancer. Cancer Res 54:2098–2101.

72. Miller DG (1980): On the nature of susceptibility to cancer. Cancer 46:1307–1318.

73. Mironov NM, Aguelon MA-M, Potapova GI, Omori Y, Gorbunov OV, Klimenkov AA, Yamasaki H (1994): Alterations of $(CA)_n$ DNA repeats and tumor suppressor genes in human gastric cancer. Cancer Res 54:41–44.

74. Modrich P (1991): Mechanisms and biological effects of mismatch repair. Annu Rev Genet 25:229–253.

75. Modrich P (1994): Mismatch repair, genetic stability, and cancer. Science 266:1959–1960.

76. Morson BC, Bussey HJR, Day WD (19830: Adenomas of the large bowel. Cancer Surv 2:451–477.

77. Nicolaides NC, Papadopoulos N, Liu B, Wei Y-F, Carter CK, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Dunlop MG, Hamilton SR, Petersen MG, de la Chapelle A, Vogelstein B, Kinzler KW (1994): Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371:75–80.

78. Nyström-Lahti M, Parsons R, Sistonen P, Pylkkänen L, Aaltonen LA, Leach FS, Hamilton SR, Watson P, Bronson E, Fusaro R, Cavalieri J, Lynch J, Lanspa S, Smyrk T, Lynch P, Drouhard T, Kinzler KW, Vogelstein B, Lynch HT, de la Chapelle A, Peltomäki P (1994): Mismatch repair genes on chromosomes 2p and 3p account for a major share of hereditary nonpolyposis colorectal cancer families evaluable by linkage. Am J Hum Genet 55:659–665.

79. Nyström-Lahti M, Sistonen P, Mecklin J-P, Pylkkänen L, Aaltonen LA, Järvinen H, Weissenbach J, de la Chapelle A, Peltomäki P (1994): Close linkage to chromosome 3p and conservation of ancestral founding haplotype in hereditary nonpolyposis colorectal cancer families. Proc Natl Acad Sci USA 91:6054–6058.

79a. Palombo F, Gallinari P, Iaccarino I, Lettieri T, Hughes M, D'Arrigo A, Truong O, Hsuan JJ, Jiricny J (1995): GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. Science 268:1912–1914.

80. Papadopoulos N, Nicolaides NC, Wei Y-F, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomäki P, Mecklin J-P, de la Chapelle A, Kinzler KW, Vogelstein B (1994): Mutation of *mutL* homolog in hereditary colon cancer. Science 263:1625–1629.

80a. Papadopoulos N, Nicolaides N, Liu B, Parsons R, Lengauer C, Palombo F, D'Arrigo A, Markowitz S, Willson JKV, Kinzler KW, Jiricny J, Vogelstein B (1995): Mutations of *GTBP* in genetically unstable cells. Science 268:1915–1917.

81. Parsons R, Li G-M, Longley MJ, Fang W-H, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P (1993): Hypermutability and mismatch repair deficiency in RER$^+$ tumor cells. Cell 75:1227–1236.

81a. Parsons R, Li G-M, Longley M, Modrich P, Liu B, Berk T, Hamilton SR, Kinzler KW, Vogelstein B (1995): Mismatch repair deficiency in phenotypically normal human cells. Science 268:738–740.

82. Peltomäki P, Aaltonen LA, Sistonen P, Pylkkänen L, Mecklin J-P, Järvinen H, Green JS, Jass JR, Weber JL, Leach FS, Petersen GM, Hamilton SR, de la Chapelle A, Vogelstein B (1993): Genetic mapping of a locus predisposing to human colorectal cancer. Science 260:810–812.

83. Peltomäki PT (1994): Genetic basis of hereditary nonpolyposis colorectal carcinoma (HNPCC). Ann Med 26:215–219.

84. Ponder B (1988): Gene losses in human tumours. Nature 335: 400–402.

85. Ponz de Leon M (1994): Prevalence of hereditary nonpolyposis colorectal cancer (HNPCC). Ann Med 26:209–214.

86. Ponz de Leon M, Antonioli A, Ascari A, Zanghieri G, Sacchetti C (1987): Incidence and familial occurrence of colorectal cancer and polyps in a health-care district of northern Italy. Cancer 60: 2848–2859.

87. Ponz de Leon M, Scapoli C, Zanghieri G, Sassatelli R, Sacchetti C, Barrai I (1992): Genetic transmission of colorectal cancer: Exploratory data analysis from a population-based registry. J Med Genet 29:531–538.

88. Ponz de Leon M, Benatti P, Pedroni M, Sassatelli R, Roncucci L (1993): Risk of cancer in the follow-up of families with HNPCC. Int J Cancer 55:202–207.

89. Ponz de Leon M, Sassatelli R, Benatti P, Roncucci L (1993): Identification of hereditary nonpolyposis colorectal cancer in the general population. Cancer 71:3493–3501.

90. Presciuttini S, Varesco L, Sala P, Gismondi V, Rossetti C, Bafico A, Ferrara GB, Bertario L (1994): Age of onset in familial adenomatous polyposis: Heterogeneity within families and among *APC* mutations. Ann Hum Genet 8:331–342.

91. Prosser J, Porter D, Coles C, Condie A, Thompson AM, Chetty U, Steel CM, Evans HJ (1992): Constitutional p53 mutation in a non-Li-Fraumeni cancer family. Br J Cancer 65:527–528.
92. Pykett MJ, Murphy M, Harnish PR, George DL (1994): Identification of a microsatellite instability phenotype in meningiomas. Cancer Res 54:6340–6343.
93. Rhyu M-G, Park W-S, Meltzer SJ (1994): Microsatellite instability occurs frequently in human gastric carcinoma. Oncogene 9:29–32.
94. Rideout WM, Coetzee GA, Olumi AF, Jones PA (1990): 5-methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science 249:1288–1290.
95. Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT, Boyd J (1993): Genetic instability of microsatellites in endometrial carcinoma. Cancer Res 53:5100–5103.
96. San Jose BA, Navarro NS, Doble F (1989): Hereditary nonpolyposis colorectal cancer: An awareness. JMMS 25:37–38.
97. Scapoli C, Ponz de Leon M, Sassatelli R, Benatti P, Roncucci L, Collins A, Morton NE, Barrai I (1994): Genetic epidemiology of hereditary non-polyposis colorectal cancer syndromes in Modena, Italy: Results of a complex segregation analysis. Ann Hum Genet 58:275–295.
98. Schaaper RM, Radman M (1989): The extreme mutator effect of *Escherichia coli* mutD5 results from saturation of mismatch repair by excessive DNA replication errors. EMBO J 8:3511–3516.
99. Scott N, Quirke P (1993): Molecular biology of colorectal neoplasia. Gut 34:289–292.
100. Shibata D, Peinado MA, Ionov Y, Malkhosyan S, Perucho M (1994): Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. Nat Genet 6:273–281.
101. Shridhar V, Siegfried J, Hunt J, del Mar Alonso M, Smith DI (1994): Genetic instability of microsatellite sequences in many non-small cell lung carcinomas. Cancer Res 54:2084–2087.
102. Steichen-Gersdorf E, Gallion HH, Ford D, Girodet C, Easton DF, DiCioccio R, Evans G, Ponder MA, Pye C, Mazoyer S, Noguchi T, Karengueven F, Sobol H, Hardouin A, Bignon Y-J, Piver S, Smith SA, Ponder BAJ (1994): Familial site-specific ovarian cancer is linked to BRCA1 on 17q12–21. Am J Hum Genet 55:870–875.
103. Strand M, Prolla TA, Liskay RM, Petes TD (1993): Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature 365:274–276.
104. Thibodeau SN, Bren G, Schaid D (1993): Microsatellite instability in cancer of the proximal colon. Science 260:816–819.
105. Umar A, Boyer JC, Thomas DC, Nguyen DC, Risinger JI, Boyd J, Ionov Y, Perucho M, Kunkel TA (1994): Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J Biol Chem 269:14367–14370.
106. Vasen HFA, den Hartog Jager FC, Menko FH, Nagengast FM (1989): Screening for hereditary nonpolyposis colorectal cancer: A study of 22 kindreds in the Netherlands. Am J Med 86:278–281.
107. Vasen HFA, Offerhaus GJ, den Hartog Jager FC, Menko FH, Nagengast FM, Griffioen G, van Hogezand RB, Heintz AP (1990): The tumour spectrum in hereditary nonpolyposis colorectal cancer: A study of 24 kindreds in the Netherlands. Int J Cancer 46:31–34.
108. Vasen HFA, Mecklin J-P, Meera Khan P, Lynch HT (1991): The International Collaborative Study Group on Hereditary Nonpolyposis Colorectal Cancer. Dis Colon Rectum 34:424–425.
109. Vogelstein B, Kinzler KW (1993): The multistep nature of cancer. Trends Genet 9:138–141.
110. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smiths AKM, Bos JL (1988): Genetic alterations during colorectal tumor development. N Engl J Med 319:525–532.
111. Warthin AS (1913): Heredity with reference to carcinoma. Arch Intern Med 12:546–555.
112. Watson P, Lynch HT (1993): Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 71:677–685.
113. Westlake PJ, Bryant HE, Huchcroft SA, Sutherland LR (1991): Frequency of hereditary nonpolyposis colorectal cancer in southern Alberta. Dig Dis Sci 36:1441–1447.
114. Wooster R, Cleton-Jansen A-M, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BAJ, von Deimling A, Wiestler OD, Cornelisse CJ, Devilee P, Stratton MR (1994): Instability of short tandem repeats (microsatellites) in human cancers. Nat Genet 6:152–156.
115. Yee CJ, Roodi N, Verrier CS, Parl FF (1994): Microsatellite instability and loss of heterozygosity in breast cancer. Cancer Res 54:1641–1644.